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Reduction of chromate by fixed films of sulfate-reducing bacteria using hydrogen as an electron source

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The ability of sulfate-reducing bacteria (SRB) to reduce chromate, Cr(VI), was evaluated using fixed-film growth systems and H_2 as the electron source. A main objective of the experiment was to distinguish between direct enzymatic reduction and indirect reduction by hydrogen sulfide, in order to subsequently verify and control the synergy of these two mechanisms. In batch experiments with the sulfate-reducing consortium CH10 selected from a mining site, 50 mg l^{-1} Cr(VI) was reduced in 15 min in the presence of 500 mg l^{-1} hydrogen sulfide compared to 16 mg l^{-1} reduced in 1 h without hydrogen sulfide. Fixed films of a CH10 population and *Desulfomicrobium norvegicum* were fed-batch grown in a column bioreactor. After development of the biofilm, hydrogen sulfide was removed and the column was fed continuously with a 13-mg l^{-1} Cr(VI) solution. Specific Cr(VI) reduction rates on pozzolana were close to 90 mg Cr(VI) h^{-1} per gram of protein. Exposure to Cr(VI) had a negative effect on the subsequent ability of CH10 to reduce sulfate, but the inhibited bacteria remained viable.
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Introduction

Chromium may exist in a number of oxidation states, but the most stable and common forms are trivalent chromium, Cr(III), and hexavalent chromium, Cr(VI). The properties of these chemical species are sharply contrasting; Cr(VI) is an oxyanion (chromate), mobile in the pore water of soils, and the more toxic form, whereas Cr(III) is much less mobile, adsorbs to particles more strongly and is decreasingly soluble above pH 4 with complete precipitation above pH 5.5 [12]. Cr(VI) is toxic, mutagenic and carcinogenic [6,14]. Cr(III), in contrast, is much less toxic and plays a physiological role in mammals by participating in glycaemia regulation [18]. Any method that facilitates the reduction of Cr(VI) into Cr(III) could be important for the treatment of industrial or mining effluents, soils and ground waters.

During the last decade, Cr(VI)-reducing bacterial strains or populations have been considered with a view to industrial applications [2,5,16,22,23,24,25]. These microorganisms reduce Cr(VI) directly to Cr(III) under either anaerobic [22] or aerobic [25] conditions. When growth is anaerobic, the reduction might result in respiration [20], Cr(VI) resistance, or may be a nonspecific enzymatic reaction. Cr(VI) reduction rates as high as 260 mg l^{-1} h^{-1} have been obtained [24]. However, all these microorganisms obtain energy from oxidation of organic substrates. In contrast, some sulfate-reducing bacteria (SRB) are able to reduce Cr(VI) using H_2 as the electron source [8,21]. Moreover, they may be able to grow in partial chemolithotrophic conditions, consuming a minimum amount of acetate as a complementary carbon source [1]. This suggests that a process using SRB to reduce Cr(VI) would generate a minimum of organic waste as dissolved matter and sludge. In addition, by reducing sulfate, SRB generate hydrogen sulfide (H_2S, HS^- and S^{2-}) which is an efficient Cr(VI)-reducing chemical; Cr(VI) is converted into Cr(III), then precipitates. Such a chemical process has been proposed for treating wastewater from industrial electroplating [26].

The simple indirect reduction of Cr(VI) with H_2S, using fermentative SRB, was patented in 1991 [11]. In order to minimize costs and waste products, it appears appropriate to consider combining the enzymatic and chemical methods into a system of Cr(VI) reduction involving chemolithotrophic SRB. The first step is to evaluate and optimize the mechanism of direct reduction independently from the indirect H_2S reaction. This paper describes experiments aiming to quantify the direct enzymatic Cr(VI)-reducing activity of SRB grown as fixed films with H_2, CO_2, and acetate.

Materials and methods

Bacterial populations

The CH10 sulfate-reducing population originated from a disused gold mine at Cheni (Limousin, France). Samples from this site were used to inoculate sealed bottles containing an SRB enrichment medium (kits Labège®, Elf-Aquitaine, France). The chemotrophic population was selected by subculturing one of these enrichment cultures with H_2 as the sole energy source and with CO_2 and acetate as the only carbon sources. CH10 is composed of mobile vibrio- and rod-shaped bacteria. The *Desulfomicrobium norvegicum* type strain Norway 4 was obtained from NCIMB (strain 8310).

Culture media

CH10 was cultured routinely in industrial medium with urea (IMU, pH 6.8) containing, in tap water, 0.21 g l^{-1} urea (nitrogen >46.2%; Brenntag, St Cyr en Val, France), 0.23 g l^{-1} diaminophosphate

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(DAP: NH_3 25.5%, PO_4^{3-} 72.5%; Brenntag) fertilizer, 0.4 g l^{-1} $MgCl_2{\cdot}6H_20$, 0.25 g l^{-1} KOH, 0.5 g l^{-1} Na_2CO_3, 7.1 g l^{-1} Na_2SO_4, 0.5 g l^{-1} sodium acetate, 0.5 ml l^{-1} of 1 g l^{-1} rezasurin solution, and 1 ml l^{-1} trace metals solution whose composition was 3 g l^{-1} EDTA, 1.1 g l^{-1} $FeSO_4{\cdot}7H_2O$, 65 mg l^{-1} $MnSO_4$, 89 mg l^{-1} $ZnSO_4$, 24 mg l^{-1} $NiCl_2$, 18 mg l^{-1} $Na_2MoO_4{\cdot}2H_2O$, 0.3 g l^{-1} H_3BO_3, 2 mg l^{-1} $CuCl_2$ and 130 mg l^{-1} $CoSO_4{\cdot}7H_2O$. Cultures were prepared in 500-ml bottles containing 100 ml liquid, with H_2/CO_2 (95%/5%) at 1.6 bar above atmospheric pressure in the headspace, and incubated at 31°C. All experiments with CH10 were performed in non-aseptic conditions. *D. norvegicum* was grown routinely on a lactate–sulfate medium (pH 7.2) containing, in deionized water, 2 g l^{-1} NH_4Cl, 2 g l^{-1} $MgSO_4{\cdot}7H_2O$, 4 g l^{-1} Na_2SO_4, 0.5 g l^{-1} K_2HPO_4, 1 g l^{-1} yeast extract (Sigma, St. Louis, MO), 9 ml of 60% sodium lactate solution, and 1 ml l^{-1} trace metals solution as above. Cultures were performed in 250-ml serum bottles at 37°C under N_2 atmosphere (0.5 bar above atmospheric pressure). All experiments with *D. norvegicum* were performed under aseptic conditions.

Cr(VI) reduction in batch cultures

Samples of pozzolana, a volcanic basaltic ash material, were kindly supplied by the quarry Carrière de Denise, Le Puy, France. Pozzolana contains (analysis by X-ray diffractometry): plagioclase $(Na,Ca)Al(SI,Al)Si_2O_8$; clinopyroxene $Mg_2Si_2O_6$–$(Fe,Mg)Si_2O_6$; hematite (Fe_2O_3)–goethite FeO(OH), and a hydrated Ca–Fe nitrate. The pozzolana pieces, size 2–5 mm, were thoroughly rinsed with tap water then dried for 24 h at 50°C. Reduction of Cr(VI) under H_2/CO_2 atmosphere was tested under the following conditions: (1) pozzolana with water and sulfide; (2) biofilm on pozzolana with water and sulfide; (3) biofilm on pozzolana with the culture medium containing sulfide but no free suspended bacteria; and (4) biofilm on pozzolana with water. In condition (1), 500-ml bottles were partly filled with 72 g of fresh pozzolana and 100 ml of deoxygenated deionized water. For conditions (2), (3), and (4), a series of 500-ml bottles were partly filled with 72 g of pozzolana and 100 ml of IMU. These bottles were inoculated with 10 ml of a CH10 culture (end of the growth phase, $2–3\times10^8$ bacteria ml^{-1}), then incubated for a 6-day period. At the end of the growth phase (final bacterial density $2–3\times10^8$ bacteria ml^{-1}), the liquid with freely suspended bacteria was removed with a syringe and the pozzolana in the bottles was rinsed three times with 100 ml deoxygenated demineralized water in order to remove the dissolved hydrogen sulfide. The dissolved sulfide concentrations were analyzed in culture media and rinse-water samples. In condition (2), the bottles containing pozzolana with fixed bacteria were injected with 100 ml of a Na_2S solution containing 500 mg l^{-1} hydrogen sulfide in deoxygenated deionized water. In condition (3), the liquid cultures from the flasks were centrifuged for 20 min at 10,000 rpm under a N_2 atmosphere in order to remove bacteria, then the supernatant was re-injected into the bottles. In condition (4), the bottles containing pozzolana with fixed bacteria were injected with 100 ml of deoxygenated deionized water. The pH of the solutions was controlled in all flasks, and adjusted if necessary to 7.5–8.0 by injecting HCl or NaOH. The headspaces of all bottles were filled with H_2/CO_2 (95%/5%) at 1.6 bar followed by the injection of 0.5 ml of a 10 g l^{-1} Cr(VI) solution prepared with Na_2CrO_4 into each bottle. The bottles were incubated at 31°C and samples were taken for Cr(VI) quantification.

Fed-batch growth in column reactors

Cultures were developed in a glass column bioreactor of 500-ml inner volume, 45-mm diameter and 330-mm height (Figure 1A). The column was filled with pozzolana, which reduced the working volume to 450 ml. IMU was inoculated with 30 ml of CH10 or *D. norvegicum* cultures at the end of the exponential growth phase ($2–3\times10^8$ bacteria ml^{-1}), and the gas phase (9.4 l h^{-1} H_2+0.4 l h^{-1} CO_2) was injected at the bottom. The temperature was 31°C, liquid culture was circulated by up-flow at 10 ml h^{-1} and the outgoing gas was bubbled into a 50-g l^{-1} zinc acetate solution. For the experiment with *D. norvegicum*, the filled column was autoclaved previously at 120°C three times at 24-h intervals. When the sulfate was entirely consumed, half the liquid content of the reactor was replaced by fresh IMU. This fed-batch procedure carried on until the culture was 300 h old.

Continuous Cr(VI) reduction in column reactors

When the Cr(VI) reduction experiments started, the cultures were in an active phase, i.e., the last pool of fed sulfate was not entirely consumed. Gas (H_2+CO_2) feeding and regulation of temperature at 31°C were maintained. The first step was the elimination of dissolved sulfide; (1) the liquid content of the column was pumped out by down-flow, (2) the support was drained off for 10 min, (3) a NaCl 5 g l^{-1} solution in deoxygenated deionized water was pumped up-flow into the column at 950–1000 ml h^{-1}. This first rinse operation was continued until the hydrogen sulfide concentration was maintained at lower than 1 mg l^{-1} for 30 min. Then, the Cr(VI) reduction step commenced: (1) the liquid content of the column was pumped out down-flow, (2) the support was drained off for 10 min, (3) a solution containing 5 g l^{-1} NaCl and 13 mg l^{-1} Cr(VI) as Na_2CrO_4 in deoxygenated deionized water was pumped up-flow into the bioreactor at 480–500 ml h^{-1} (Figure 1B). Samples for Cr(VI) analysis were taken from the liquid upper exit at 10-min intervals throughout a period of 120 min.

The second rinse operation aimed to quantify the dilution of Cr(VI) in the first rinse solution retained by the support: (1) the liquid content of the column was pumped out down-flow, (2) the support was drained off for 10 min, (3) deionized water was pumped up-flow into the bioreactor at a flow rate strictly identical

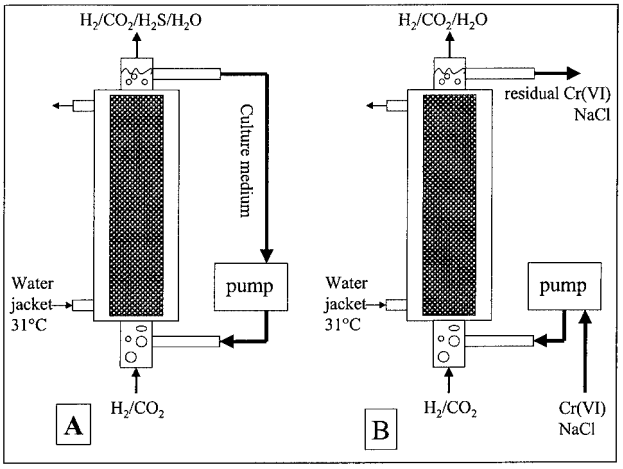

Figure 1 Experimental device: (A) column bioreactor during the biofilm growth phase in fed batch; (B) column configuration during the Cr(VI) reduction test.

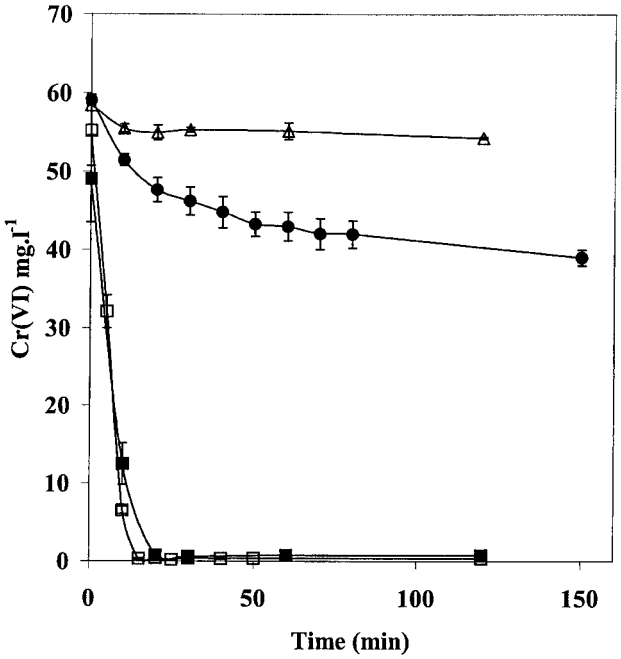

Figure 2 Batch reduction of Cr(VI) by CH10 fixed on pozzolana support: (Δ) blank pozzolana+water+H_2 with neither bacteria nor hydrogen sulfide, (■) pozzolana+water+H_2+biofilm+hydrogen sulfide, (□) pozzolana+culture medium containing sulfide+biofilm (●) pozzolana+H_2+biofilm. Error bars represent the standard error of the mean (three replicates).

to that used previously for the Cr(VI) feeding. Samples for sodium analysis were taken at 10-min intervals from the liquid upper exit. The dilution factor of Cr(VI) in the first rinse solution was calculated using the decrease of Na concentration during the second rinse: $D=1-([\mathrm{Na}]t/[\mathrm{Na}]t_0)$ where D=Cr(VI) dilution factor at time t.

A Cr(VI) reduction experiment to quantify the loss of Cr(VI) by adsorption or nonbiological reduction was undertaken using noncolonized pozzolana. In order to evaluate the viability of the CH10 population a first step rinse and Cr(VI) exposure, the column was filled with culture medium and normal growth operating conditions were applied subsequently to each of the above two operations.

Analyses

Fresh bacterial cultures were observed and enumerated under an optical microscope (400× magnification). In the batch experiments, Cr(VI) was quantified using the kit Spectroquant® 1.14758.0001 (Merck Eurolab, Fontenay-sous-bois, France), based on the oxidation of diphynelcarbazide to the purple-colored Cr(III)–diphynelcarbazone complex (detection limit 0.025 mg l^{-1}). In continuous experiments, Cr(VI) was diluted in a borate buffer (pH 9.5) and quantified spectrophotometrically (HP 8452A Hewlett-Packard, Les Ulis, France) at 370 nm (detection limit 0.1 mg l^{-1}). Total chromium and sodium were analyzed by atomic absorption spectrophotometry (Varian Les Ulis, France SpectrAA-300). Total dissolved sulfide was determined by potentiometric titration (TIM 900 Titralab Radiometer Analytical, Villeurbanne, France) with $HgNO_3$ (5×10^{-4} M), using a specific sulfide Ag–Ag_2S electrode coupled to a reference Ag–AgCl electrode. In order to avoid oxidation, syringes were totally filled with sample and analyses were carried out immediately. Sulfate was quantified using the kit Merck 1.14548.001 (detection limit 5 mg l^{-1}). Proteins were extracted from the fixed film by heating the support in 1 N NaOH to ebullition for 10 min and then analyzed in the supernatant using the Lowry method [10]. Analyses of fresh (blank) pozzolana samples showed positive responses. The positive values of the pozzolana blanks were subtracted from the response of bacteria-colonized samples.

Results

Batch experiments

When the pozzolana was rinsed in order to eliminate hydrogen sulfide, the dissolved sulfide concentration in the third rinse water was always less than 1 mg l^{-1} (0.03 mM). Cr(VI) reduction in the presence of 500 mg l^{-1} dissolved hydrogen sulfide (15 mM, which is a common level of concentration in CH10 batch cultures), was rapid: 50 mg l^{-1} Cr(VI) was reduced during the first 15 min of the experiment (Figure 2). Similar results were obtained with culture media containing biologically produced hydrogen sulfide, and Na_2S dissolved in deionized water. In the absence of hydrogen sulfide, the fixed bacteria reduced Cr(VI) at a slower rate: 16 mg l^{-1} Cr(VI) was reduced in 1 h, and the Cr(VI) reduction continuously slowed down. Tests showed insignificant reduction of Cr(VI) by H_2 in the presence of pozzolana alone without bacteria or sulfide.

Experiments using columns

Growth during an experiment with CH10 and pozzolana support is shown in Figure 3. The successive increases in sulfate concentration result from injections of fresh medium according to the fed-batch culture method. Reduction of SO_4^{2-} with H_2 consumes acidity [7], but an equilibrium is obtained though dissolution of CO_2 from the feeding gas. Neither the removal of free suspended

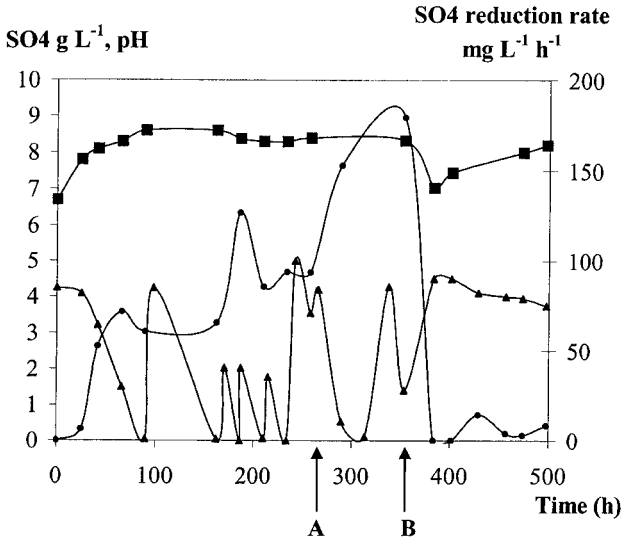

Figure 3 Behaviour of the CH10 population grown in fed-batch condition as a fixed film on pozzolana in the column bioreactor: (■) pH, (▲) SO_4^{2-} g l^{-1}, (●) sulfate reduction rate, mg l^{-1} h^{-1}. A: One rinse with NaCl 5 g l^{-1} followed by normal growth conditions; B: complete Cr(VI) reduction experiment followed by normal growth conditions. The successive increases in SO_4 concentration correspond to additions of fresh medium.

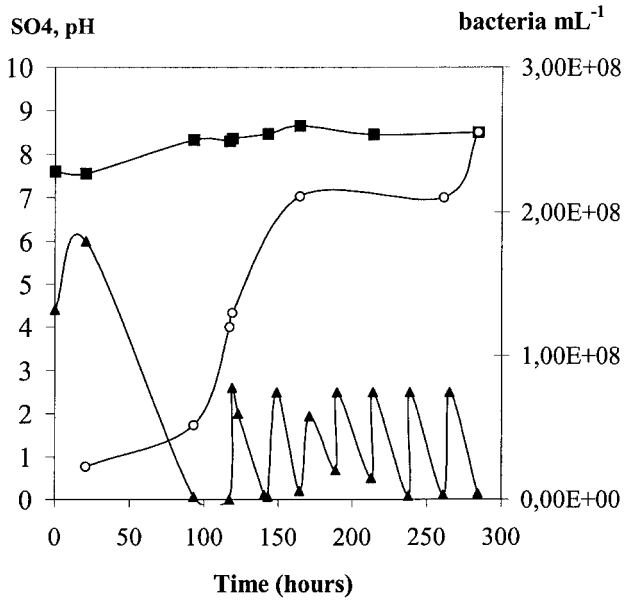

Figure 4 Behaviour of *D. norvegicum* grown in fed-batch condition as a fixed film on pozzolana in the column bioreactor: (■) pH, (▲) SO_4^{2-} g l^{-1}, (○) suspended bacteria enumerated under an optical microscope (Thoma cell). The successive increases in SO_4 concentration correspond to additions of fresh medium.

bacteria nor a first rinse of the support with a NaCl solution (5 g l^{-1}) affected the fixed film. On the contrary, the SO_4^{2-} reduction rate was higher after the rinse operation, reaching 180 mg l^{-1} h^{-1}, which is close to the maximum value obtained previously with the same bacterial population [4]. Conversely, the Cr(VI) reduction test had a marked negative effect on the SO_4^{2-} reduction rate, which decreased to 20 mg l^{-1} h^{-1}. However, the fixed film was not entirely killed by exposure to Cr(VI). The concentration of free

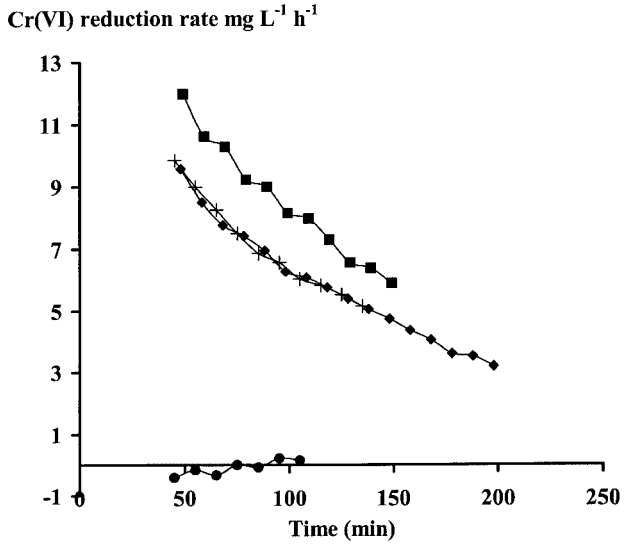

Figure 5 Evolution of Cr(VI) reduction rate during the continuous feeding test. Rates were calculated as follows: [Cr(VI) feed − D×Cr(VI) exit]× feed flow rate/reactor working volume (D=dilution factor). (●) Pozzolana without bacteria, (◆) pozzolana+CH10 experiment 1, (+) pozzolana+CH10 experiment 2 (total mass of proteins 51 mg), (■) pozzolana+*D. norvegicum* (total mass of proteins 50 mg). Origin of time: start of continuous Cr(VI) feeding.

Table 1 Maximum reduction specific rates obtained in the present experiments compared to previously published results

Organism	Maximum reduction specific rate [mg Cr(VI) h^{-1} per gram of proteins]
CH10 on pozzolana (H_2)	80
Desulfomicrobium norvegicum on pozzolana (H_2)	90
Desulfomicrobium norvegicum, free cells (lactate [13])	100
Desulfovibrio vulgaris, free cells (H_2 [9])	80
Desulfovibrio desulfuricans, free cells (lactate [21])	25
Desulfovibrio desulfuricans, immobilised in polyacrylamide [21] H_2	5
lactate	4

suspended motile bacteria increased up to 10^8 ml^{-1}, and a rise of pH was observed.

One experiment in column was carried out with *D. norvegicum*, which was selected for its high Cr(VI)-reducing activity as entire cells and purified cytochrome c_3 [13]. *D. norvegicum* grew chemotropically without any problem (Figure 4), in accordance with its physiological phenotype [17]. The inoculum, prepared in lactate–sulfate medium, may have introduced some residual nutrients, but these were consumed and diluted during the successive fed-batches and the bacteria retained their ability to reduce sulfate. Examination of fresh samples under an optical microscope revealed that the *Desulfomicrobium*-inoculated column contained only motile rod-shaped bacteria in contrast to the CH10 population, which was a mixture of rod- and vibrio-shaped organisms.

The evolution of Cr(VI) reduction rates in continuous feeding conditions is shown in Figure 5. The total chromium concentration in the samples was always equal to the Cr(VI) concentration: the amount of Cr(III) in the liquid upper exit of the column was negligible in comparison with Cr(VI) concentration. A slowing down of Cr(VI) reduction over time was observed. The whole experiment was repeated with CH10, and results of the two tests were identical. The *D. norvegicum* biofilm was slightly more efficient than CH10 for Cr(VI) reduction. The total mass of proteins in the column was 50 mg with CH10 and 51 mg with *D. norvegicum*. The maximum specific Cr(VI) reduction rates are compared in Table 1 with published results.

Discussion

Two hypotheses can be proposed to explain the absence of Cr(III) in the effluent of the continuously fed column: (1) biosorption of Cr(VI), and (2) sorption and/or precipitation of Cr(III) on pozzolana. Biosorption of chromate has been shown to occur [8]. However, with SRB, the enzymatic reduction process was shown to be preponderant [9,13]. Reduction of Cr(VI) by entire cells and by purified enzymes from *D. norvegicum* were correlated [13]. When *D. norvegicum* cells were cultivated in the absence of mineral support, colloidal and dissolved Cr(III) could be detected in the liquid medium (unpublished data). Moreover, in batch conditions, the absence of Cr(III) in the liquid phase was also recorded when Cr(VI) was chemically reduced by hydrogen sulfide in the presence of pozzolana. Later, deposition of the blue-green

precipitate of Cr(III) hydroxide on pozzolana was observed when reduction of Cr(VI) in presence of hydrogen sulphide was performed in continuous conditions (unpublished data). Smith and Gadd [19] showed that a biofilm of a mixed sulfate-reducing bacterial population cultivated on lactate was able to reduced 90 μM Cr(VI), and that the biofilm itself retained less than 10% of the total chromium. Tucker *et al* [21] also found that sorption of chromium of immobilized cells of *Desulfovibrio desulfuricans* was insignificant.

The slowing down of Cr(VI) enzymatic reduction under batch conditions has been observed frequently with pure enzymes [9] or entire cells [21] of SRB. This phenomenon may result solely from a decrease in Cr(VI) concentration (i.e., Cr(VI) considered as the enzyme "substrate" [15]) or, alternatively, from a combination of both Cr(VI) concentration decrease and inhibition [27]. In continuous conditions, the decrease of Cr(VI) reduction rate results from inhibition of inactivation of the enzymes. During the experiment with continuous feed containing 13 mg l^{-1} Cr(VI), the Cr(VI) reduction process was inactivated in a few hours. The enzymes responsible for Cr(VI) reduction were probably inactivated, and could not be synthesized as the bacteria were in nongrowing conditions. Tebo and Obraztsova [20] found that *Desulfotomaculum reducens* MI-1 can use Cr(VI) as electron acceptor, but Cr (VI) concentrations greater than 200 μM (10 mg l^{-1}) inhibited growth of this strain. In the present experiment, although the rinse operation by itself had no apparent influence on the activity of the biofilm, exposure to Cr(VI) inhibited not only the Cr(VI)-reducing process, but also the subsequent ability of the cells to reduce SO_4^{2-}. Chromate can act as an analogue of sulfate by virtue of its chemical similarity, and is a competitive inhibitor of sulfate transport in *Pseudomonas* [3]. Sulfate reduction by the biofilm of SRB studied by Smith and Gadd [19] was also markedly inhibited by 100 μM (5 mg l^{-1}) Cr(VI) may have damaged some cellular components involved in SO_4^{2-} uptake and respiration. However, this inhibited state is reversible.

CH10 and *D. norvegicum* exhibited similar abilities to grow as a biofilm on pozzolana, with similar amounts of proteins attached to the support after 300 h of fed-batch culture. The Cr(VI) reduction rate was slightly higher with *D. norvegicum*; it was reported that this strain displays particular efficiency in Cr(VI) reduction [13]. In terms of specific rate, the performances of CH10 and *D. norvegicum* with H_2 on pozzolana (around 90 mg Cr(VI) h^{-1} per gram protein), are close to those of *D. norvegicum* as free cells with lactate [13], and *Desulfovibrio desulfuricans* as free cells with H_2 [9]. The specific activity of the cells is likely to decrease when the thickness of the biofilm increases. Immobilization of *Desulfovibrio desulfuricans* in polyacrylamide had a negative effect on Cr(VI) reduction rate [21], as transport limitations may control the process. In chemotrophic biofilm reactors, limitation problems might be avoided by use of H_2-fluidised bed systems, which would also limit the accumulation of chromium hydroxide. By itself, enzymatic Cr(VI) reduction by SRB is not competitive with the organotrophic Cr(VI) populations described by Turick *et al* [24] and Shakoori *et al* [16], because these authors obtained Cr(VI) reduction rates as high as 260 mg l^{-1} h^{-1}. However, addition of sulfate to the feed may greatly improve the kinetics of Cr(VI) reduction and precipitation, as suggested by the results of the batch experiments.

The synergy of direct and indirect mechanisms and the selection of strains displaying high enzymatic reduction rates may allow development of a cleaner process. In this perspective, the ability of *D. norvegicum* to grow easily in chemotrophic industrial media is encouraging.

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References

1 Badziong W and RK Thauer. 1978. Growth yields and growth rates of *Desulfovibrio vulgaris* (Marburg) growing on hydrogen plus sulfate and hydrogen plus thiosulfate as the sole energy sources. *Arch Microbiol* 117: 209–214.
2 Bhide JV, PK Dhakephalkar and KM Paknikar. 1996. Microbiological process for the removal of Cr(VI) from chromate-bearing cooling tower effluent. *Biotechnol Lett* 18: 667–672.
3 Cervantes C. 1991. Bacterial interaction with chromate. *Antonie van Leeuwenhoek* 59: 229–233.
4 Foucher S, F Battaglia-Brunet, I Ignatiadis and D Morin. 2000. Treatment by sulfate-reducing bacteria of Chessy acid-mine drainage and metals recovery. *Chem Eng Sci* 56: 1639–1645.
5 Gourdon R, P Diard and N Funtowicz. 1999. Effect of microbial activity on the mobility of chromium in soils. In Méhu J, G Keck and A Navarro, (Eds), Waste Stabilization and Environment Proceeding Actes. La Société Alpine de Publications, Grenoble, pp. 153–157.
6 Gruber JE and KW Jennette. 1978. Metabolism of the carcinogen chromate by rat liver microsomes. *Biochem Biophys Res Commun* 82: 700–706.
7 Herrera L, J Hernandez, L Bravo, L Romo and L Vera. 1997. Biological process for sulfate and metals abatement from mine effluents. *Environ Toxicol Water Qual* 12: 101–107.
8 Lee DC, CJ Park, JE Yang, YH Jeong and HI Rhee. 2000. Screening of hexavalent chromium biosorbent from marine algae. *Appl Microbiol Biotechnol* 54: 597–600.
9 Lovley DR and EJP Phillips. 1994. Reduction of chromate by *Desulfovibrio vulgaris* and its c_3 cytochrome. *Appl Environ Microbiol* 60: 726–728.
10 Lowry OH, NJ Rosebrough, AL Farr and RJ Randall. 1951. Protein measurement with the folin–phenol reagents. *J Biol Chem* 193: 265–275.
11 Lupton FS, LJ DeFilippi and JR Goodman. 1991. Bioremediation of chromium (VI) contaminated aqueous systems by sulfate reducing bacteria. US patent no. 5,062,956.
12 McGrath SP and S Smith. 1993. Chromium and nickel. In Heavy metals in soils BJ Alloway, (Ed), Blackie Academic & Professional, Glasgow, UK, pp. 124–150.
13 Michel C, M Brugna, C Aubert, A Bernadac and M Bruschi. 2001. Enzymatic reduction of chromate: comparative studies using sulfate-reducing bacteria. Key role of polyheme cytochromes *c* and hydrogenases. *Appl Microbiol Biotechnol* 55: 95–100.
14 Petrilli FL and S de Flora. 1977. Toxicity and mutagenicity of hexavalent chromium in *Salmonella typhimurium*. *Appl Environ Microbiol* 33: 805–809.
15 Schieman EA, DR Yonge, MA Rege, JN Petersen, CE Turick, DL Johnstone and WA Apel. 1998. Comparative kinetics of bacterial reduction of chromium. *J Environ Eng* 124: 449–455.
16 Shakoori AR, S Tahseen and RU Haq. 1999. Chromium-tolerant bacteria isolated from industrial effluents and their use in detoxication of hexavalent chromium. *Folia Microbiol* 44: 50–54.
17 Sharak Genthner BR, G Mundfrom and R Devereux. 1994. Characterization of *Desulfomicrobium escambium* sp. nov. and proposal to assign *Desulfovibrio desulfuricans* strain Norway 4 to the genus *Desulfomicrobium*. *Arch Microbiol* 161: 215–219.

18 Shroeder HA. 1968. The role of chromium in mammalian nutrition. *Am J Clin Nutr* 21: 230–244.
19 Smith WL and GM Gadd. 2000. Reduction and precipitation of chromate by mixed culture sulfate-reducing bacterial biofilms. *J Appl Microbiol* 88: 983–991.
20 Tebo BM and AY Obraztsova. 1998. Sulfate-reducing bacterium grows with Cr(VI), U(VI), Mn(IV), and Fe(III) as electron acceptors. *FEMS Microbiol Lett* 162: 193–198.
21 Tucker MD, LL Barton and BM Thomson. 1998. Reducing of Cr, Mo, Se and U by *Desulfovibrio desulfuricans* immobilized in polyacrylamide gels. *J Ind Microbiol Biotechnol* 20: 13–19.
22 Turick CE and WA Apel. 1997. A bioprocessing strategy that allows for the selection of Cr(VI) bacteria from soils. *J Ind Microbiol Biotechnol* 18: 247–250.
23 Turick CE and WA Apel. 1997. Method for *in situ* or *ex situ* bioremediation of hexavalent chromium contaminated soils and/or groundwater. US Patent no. 5,681,739.
24 Turick CE, CE Camp and WA Apel. 1997. Reduction of Cr(6+) to Cr(3+) in a packed-bed bioreactor. *Appl Biochem Biotechnol* 63–65: 871–877.
25 Wang YT and EMN Chirwa. 1996. Bioremediation of chromium by packed-bed bioreactors. In Weber AS, (Ed), Proceedings of the twenty-eighth Mid-Atlantic Industrial and Hazardous Waste Conference. Technomic Publishers, Lancaster, pp. 381–388.
26 Whang JS, D Young and M Pressman. 1982. Soluble-sulfide precipitation for heavy metals removal from wastewaters. *Environ Progr* 1: 110–113.
27 Yamamoto K, J Kato, T Yano and H Ohtake. 1993. Kinetics and modeling of hexavalent chromium reduction in *Enterobacter cloacae*. *Biotechnol Bioeng* 41: 129–133.